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The Late Pleistocene distribution of vicuñas (*Vicugna vicugna*) and the "extinction" of the gracile llama ("*Lama gracilis*"): New molecular data

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ABSTRACT

We present the results of a palaeogenetic analysis of two Late Pleistocene camelids originating in southern Chile. Our analysis of two mitochondrial DNA fragments (control region and cytochrome *b* gene) reveals that these specimens do not belong to an extinct taxon, but rather to extant vicuña (*Vicugna vicugna*), whose modern distribution is restricted to the extreme elevations of the Andes, more than 3500 km to the north of where these specimens originated. Our results also suggest fossil specimens from Patagonia that are currently assigned to the extinct taxon *Lama gracilis*, may actually belong to *V. vicugna*, implying a continuous distribution of the latter from the southern tip of South America to the Andes during the Final Pleistocene. The haplotypes of both specimens are not present in modern populations, suggesting a loss of genetic diversity concomitant with the contraction of the vicuña geographical distribution during the Final Pleistocene or early Holocene.

1. Introduction

Two wild species of camelids inhabit South America at present: guanacos (*Lama guanicoe*) and vicuñas (*Vicugna vicugna*). The former inhabits a wide range of arid and semi-arid environments, from north-central Andes to Patagonia (8° to 55° S), at altitudes ranging from sea level to over 4000 m above sea level. In contrast, the modern distribution of vicuñas is restricted to the Puna of the central Andes, an area of high altitude grasslands c. 3800–4900 m above sea level between latitudes 9° 30′ and 29°S (Franklin, 1982; Wheeler, 1995; Fig. 1). During archaeological excavations in southern Chile in the 1980s and 1990s, two peculiar camelid remains were recovered in Late Pleistocene localities in southern Patagonia and Tierra del Fuego (Prieto and Canto, 1997; Massone and Prieto, 2004; Fig. 1). While both specimens had morphological characteristics resembling modern vicuñas, these locations are more than 3500 km south of, and over 3000 m lower in elevation than, the present-day habitat of the species. These remains can be explained by two alternate hypotheses: either these specimens represent a camelid species closely related to, but distinct from, the vicuña that became extinct – as many other megafauna taxa – towards the end of the Pleistocene, or they are *V. vicugna*. If the latter hypothesis is correct, the distribution of vicuñas must have been significantly more extensive during the Final Pleistocene than it is today (Prieto and Canto, 1997).

Here, we use ancient DNA (aDNA) techniques to clarify the phylogenetic status of these two anomalous camelid finds, and consider the implication of the results for the taxonomy of Late Pleistocene camelids in southern South America.

2. Materials and methods

The two camelid remains consist of a left second lower permanent incisor (I_2) from the site of Cueva Lago Sofia 4 (CLS4) in the Ultima Esperanza region, southern Patagonia, dated by accelerator mass spectrometry (AMS) to 13 100 ± 70 BP (16 010 ± 391 cal BP; P. Moreno and R. Villa, pers. comm.); and a fragmentary right

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Fig. 1. Provenance of the two camelid specimens discussed in the paper (green and red dots), as well as find-spots of reported specimens of "*Lama gracilis*" (black dots). 1. Sierra Chica, Cordoba; 2. Lujan; 3. Laguna Videl; 4. Centinela del Mar; 5. Paso Otero; 6. Los Toldos, El Ceibo, Piedra Museo; 7. Cueva del Minero (La Maria); 8. Cueva Lago Sofia 4 (green) and Cueva del Medio; 9. Tres Arroyos 1 (in red); 10 Agua de la Cueva. Sources: Menegaz et al., 1989; Borrero and Franco, 1997; Miotti and Salemme, 1999, 2003; Tauber, 1999; Paunero et al., 2004; García et al., 2008.

mandible with I_1–I_3 and canine (C_1) from Tres Arroyos 1 (TA1) in the Isla Grande of Tierra del Fuego, dated by AMS to 10 630 ± 70 BP (12 582 ± 122 cal BP; Massone and Prieto, 2004). The morphological characteristics of these specimens have been discussed previously by Prieto and Canto (1997).

2.1. Ancient DNA

DNA was extracted from pulverised samples (c. 0.1 g) of the root (I_2 in case of TA1) using established aDNA methods (reference; see also supplementary information). All extraction procedures were carried out at a dedicated aDNA facility at the Biological Institute, University of Copenhagen, physically distant to the facilities where PCR amplifications took place. Prior to this research, no previous molecular work with camelids, either ancient or modern, had been carried out at this location. A 268 base pair (bp) segment of the mitochondrial cytochrome *b* (cytb) gene was amplified for each of the samples using a series of overlapping primer sets. In addition, a 118 bp segment of the mitochondrial control region was amplified for CLS4 (details of sequences of primers as well as conditions for the PCR are presented in the supplementary information). Negative extraction controls and negative PCR controls were used to monitor possible cross-contamination, and in no case was contamination of these controls observed. Each PCR product was cloned using the Invitrogen TA cloning kit (Invitrogen, Carlsbad CA), and eight to twelve insert-containing clones were sequenced using ABI chemistry at the commercial service offered by Macrogen Inc. (Seoul, South Korea; www.macrogen.com). Cloned products showed only limited amounts of sequence modification due to template damage.

2.2. Modern DNA

Sequences for the mitochondrial control region and cytochrome *b* of modern vicuñas and guanacos from different populations across Chile, Peru and Argentina were obtained within the frame of previous studies focused on the conservation, taxonomy and domestication of South American camelids (Marin et al., 2007a,b; see these references for a full description of specimens and methods).

2.3. Phylogenetic analyses

Consensus sequences for CLS4 and TA1 were aligned manually together with those of modern guanacos and vicuñas using Se-Al v.2.0a11 (Rambaut, 2002). Phylogenetic analyses were performed using both Bayesian (MrBayes; Huelsenbeck and Ronquist, 2001) and Maximum Likekihood (ML) (PAUP v4.0b10; Swofford, 2002) statistical frameworks. The software Modeltest (Posada and Crandall, 1998) was used to select the HKY + G model of nucleotide substitution, which was used subsequently for both analyses. For the ML analysis, values for the substitution model parameters were estimated initially from a NJ tree and fixed, followed by two iterations of heuristic searching with TBR branch-swapping and re-estimation of model parameters. To valuate support for the resulting topology, 1000 bootstrap replicates were performed with resampling, with starting trees generated by NJ and TBR branch-swapping. For the Bayesian analysis, two chains were run for 5 million iterations using MrBayes default temperature settings. Mixing and convergence to stationarity were evaluated using Tracer v1.4 (Rambaut, 2007), and chains were combined following the removal of the initial 10% of iterations as burn-in. A maximum clade credibility tree and Bayesian posterior probabilities were calculated using TreeAnnotator v1.4 (Drummond and Rambaut, 2007). Fig. 2 describes the reconstructed evolutionary relationships between the guanacos and vicuñas.

3. Results and discussion

The results of the phylogenetic analysis clearly indicate a separation between modern guanacos and vicuñas (Fig. 2). The latter form two distinct subclades that largely correspond to the two modern subspecies that have been recognised on the basis of morphological characteristics: the northern *Vicugna vicugna mensalis* and the southern *Vicugna vicugna vicugna* (Marin et al., 2007a,b). In contrast to previous hypotheses, the data also demonstrates that both Pleistocene specimens CLS4 and TA1 are *V. vicugna*, rather than to a separate, extinct camelid species. Furthermore, they appear to cluster phylogenetically with *V. vicugna mensalis*, though the statistical support for this is not strong. Further molecular analysis, in particular of the rapidly evolving mitochondrial control region, will be necessary to refine this classification. Regardless of whether CLS4 and TA1 belonged to *V v. mensalis*, or to a different, now extinct subclade (subspecies), neither fossil haplotype has been found in extensive genetic studies of modern populations (Marin et al., 2007a,b, unpublished data). This indicates a loss of genetic diversity in vicuñas since the Late Pleistocene or early Holocene.

The results presented above lead to several unanswered questions about the recent evolutionary history of South American

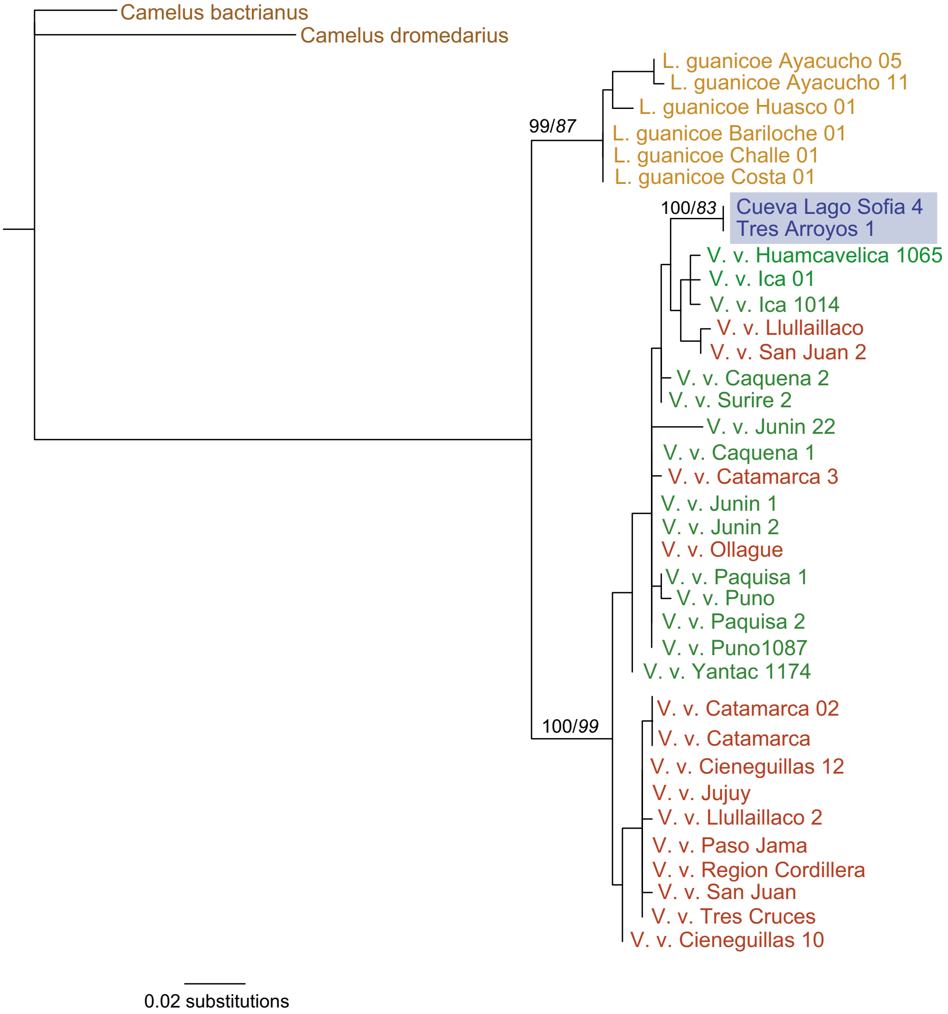

Fig. 2. Maximum likelihood phylogenetic tree of Pleistocene and modern South American camelids (HKY + G model). Brown = *Camelus bactrianus*; and *Camelus dromedarius*; Orange = Lama guanicoe; Green = *Vicugna vicugna mensalis*; Red = *Vicugna vicugna vicugna*; Blue = Pleistocene specimens CLS4 and TA1. Values above/beside nodes (in black) are posterior probabilities and ML bootstrap consensus values, respectively (only those >80% are shown). These values were generated on a reduced data set with Old World camels.

camelids. How widespread were vicuñas in the Late Pleistocene? When did they become restricted to their present high Andean habitat? Answers to these questions are likely to come from further investigation of an allegedly different camelid taxon reported from Late Pleistocene deposits in South America: the "gracile llama" *Lama* (*Vicugna*) *gracilis*. For reasons detailed below, we believe that fossil remains ascribed to this taxon are probably vicuñas, and do not represent an extinct species.

Lama gracilis was first described by Ameghino towards the end of the 19th century (Menegaz et al., 1989), and specimens assigned to this species have since been recovered from a variety of localities in Argentina and Chile (Fig. 1). In general, lower incisor teeth morphology has been considered a particularly useful diagnostic tool for discriminating between South American camelids (Menegaz et al., 1989; Wheeler, 1995). and in this regard Menegaz et al. (1989) have analysed the single available *L. gracilis* incisor at their disposal to argue its status as an intermediate between modern guanacos and vicuñas (although rather closer to the latter). However, as pointed out by Tauber (1999), some of the characteristics seen by Menegaz et al. (1989) as separating the incisors of *L. gracilis* from *V. vicugna* can either be found in some modern vicuñas as well (e.g. a thin layer of enamel on the lingual aspect), or vary during the ontogenetical development (e.g. bevelled wear facets). More importantly, another trait thought to be distinctive in *L. gracilis* – the somewhat conical shape of the incisors, as opposed to more tubular in vicuñas (Menegaz et al., 1989; Tauber, 1999) – is clearly shared by the specimen from CLS4 (see Prieto and Canto, 1997), which, as we demonstrate here, is a *V. vicugna*.

While dental (incisor) traits are considered to be the most useful diagnostic element in camelid taxonomy, the vast majority of finds assigned to *L. gracilis* consist of postcranial material (e.g. Paunero et al., 2004; Guerin and Faure, 1999). As no morphological characteristics have been found to separate the postcranial material of *L. gracilis* and *V. vicugna* – with the exception of slightly more slender metacarpals in the latter (Menegaz et al., 1989; Guerin and Faure, 1999) – the specific determination has been predominantly undertaken on the basis of size; *L. gracilis* is considered to be somewhat larger than modern vicuñas and smaller than modern guanacos. However, given that the body size of many mammals, camelids included, has been found to vary both diachronically and spatially (see e.g. Weinstock, 2000; Weinstock et al., 2005), this method is unreliable. Specifically relevant to this question, are reported changes in the size of guanacos in southern Patagonia during the Final Pleistocene and early Holocene (L'Heureaux, 2007). Thus, it could be argued that an alternative assignation of *L. gracilis* finds, i.e. as large specimens of *V. vicugna*, has been not seriously considered due to the fact that, whereas modern vicuñas are strictly orophilic, the find-spots of *L. gracilis* are situated at much lower elevations, all below 600 m above sea level. However, our results demonstrate that *V. vicugna* was present at low altitudes in southern Patagonia and Tierra del Fuego towards the end of the Pleistocene.

In light of the combined genetic and morphological arguments discussed above, we argue that the fossil remains assigned to *L. gracilis* probably belong to *V. vicugna*, as previously suggested by Guerin and Faure (1999). We further suggest that the geographical distribution of *V. vicugna* was much more extensive during the Pleistocene than it is at present, and that the species was not restricted to the extreme high elevations in which it thrives today. This hypothesis could be confirmed by genetic analysis of specimens classified as *L. gracilis*. If our hypothesis is corroborated, then radiometric dating of specimens assigned to *L. gracilis*, which is unavailable at present, could prove essential to determine the timing of the extinction of vicuñas in the extra Andean regions and the cause(s) of their demise. Radiometric dating of deposits containing remains of this taxon, dated mostly to between 11,000 and 10,000 ^{14}C BP (Ramirez Rozzi et al., 2000; Alberdi et al., 2001; Miotti and Salemme, 2003; Paunero et al., 2004; Borrero, 2008), suggests that it disappeared from Fuego-Patagonia near the Pleistocene/Holocene transition. However, specimens of *gracilis* found at level 4 of AEP1 (Piedra Museo), a stratigraphic unit from which dates of 9210 ± 105 and 9710 ± 105 ^{14}C BP, have also been reported (Miotti and Salemme, 2003). These dates suggest that vicuñas/*L. gracilis* may have been present in this region during the early Holocene.

Given that both vicuñas and guanacos were sympatric in lowland areas of southernmost South America during the Late Pleistocene, it is unclear why only the latter form part of its modern fauna.

In regions where populations of both taxa are sympatric today, guanacos coexist with vicuñas without inter-specific agonistic interactions (Cajal, 1989; Lucherini, 1996; Lucherini and Birochio, 1997). However, in comparison to the latter, guanacos possess more flexible social and foraging behaviours (González et al., 2006), which may have proved of critical importance as the climate and environment changed during the final Pleistocene and early Holocene. If local conditions deteriorate drastically, guanacos have the ability to migrate seasonally in large and mixed groups; this is in marked contrast to the all-year territoriality of vicuñas (Franklin, 1982, 1983; Lucherini, 1997). Furthermore, whereas the diet of vicuñas is restricted almost exclusively to grasses (Franklin, 1982), guanacos show a broader dietary spectrum that includes trees and shrubs (González et al., 2006), which allows them to thrive and outcompete vicuñas in all but the food-impoverished extreme elevations, to which the latter are superbly suited due to their smaller size and a series of morphological and physiological adaptations to hypoxic environments (Jürgens et al., 1988).

In the palaeontological and archaeological record of southern Patagonia and Tierra del Fuego guanacos greatly outnumber vicuñas/*L. gracilis* (e.g. Alberdi et al., 2001; Paunero et al., 2004). The establishment of modern climatic patterns at the beginning of the Holocene (Paez et al., 1999) and concomitant environmental changes probably decimated the already small vicuña populations, though humans may have also contributed to their demise: *gracilis* bones showing cut-marks made with stone tools have been uncovered at Piedra Museo (Ramirez Rozzi et al., 2000).

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.quascirev.2009.03.008.

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